The absence of the peroxiredoxin Pmp20 causes permeabilisation of the peroxisomal membrane and necrotic cell death

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PO 110

Conformational change of apolipoprotein A–I and promotion of HDL formation at acidic conditions

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The molecular mechanism by which nascent high-density lipoprotein (HDL) forms via the interaction of apolipoprotein A–I (apoA–I) and transmembrane ABCA1 is poorly understood. Here, as ABCA1 has been reported to localize to acidic intracellular compartments including the Golgi and endosome, we studied the interaction of apoA–I with model membranes in acidic conditions. Pure phosphatidylcholine (PC) liposomes were persistent against apoA–I at pH levels above 5.0, but were progressively transformed into reconstituted HDLs (rHDLs) by apoA–I at lower pH. CD and ANS fluorescence measurements of lipid-free apoA–I indicated that the accelerated formation of rHDLs was caused by the formation of α-helical structure and the increased hydrophobicity of apoA–I in acidic conditions. The addition of phosphatidylserine (PS) increased the acidity at bilayer's surface and enabled the formation of discoidal rHDLs even at the pH of the endosome and slightly lower pH of the Golgi. These results suggest a following new scenario of the nascent HDL formation; ABCA1 that colocalizes with apoA–I in the acidic intracellular compartments including the Golgi and endosome increases the acidity at the membrane's surface in the luminal side by its PS translocase activity and causes apoA–I to form nascent HDL.

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PO 111

The accumulation of two atypical sphingolipids cause hereditary sensory neuropathy type 1 (HSAN1)

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Hereditary sensory neuropathy I (HSANI) is an autosomal dominant inherited neuropathy that primarily affects peripheral sensory neurons. Patients suffer from a severe sensory loss leading to painless injuries and chronic skin ulcers. The disease is caused by several missense mutations in the *SPTLC1* gene of serine palmitoyltransferase (SPT). SPT catalyses the condensation of serine with palmitoyl-CoA—the first step in the de-novo synthesis pathway of sphingolipids.

We discovered recently that the HSN1 mutations in SPT lead to a shift in the substrate specificity of this enzyme. The mutant SPT can also metabolise alanine and glycine instead of serine as alternative substrates. The conjugation of palmitoyl-CoA with alanine or glycine results in the formation of the two atypical sphingolipids—Deoxy-sphinganine (DoxSA, m18:0) and 1-amino-2-deoxy-*n*-heptadecane (ADHD, m17:0). Hek293 cells which express the mutant form of SPTLC1 show a pronounced accumulation of these two metabolites. The absence of the C_1–OH group in DoxSA and ADHD blocks the further transformation towards complex sphingolipids (e.g. sphingomyeline or glycosphingolipids) but also prevents the degradation via the formation of Sphingosine-1P. Consequently, those "dead end" metabolites accumulate in the cells of HSN1 patient. This was confirmed by analyzing EBV lymphoblast lines from 12 HSN1 patients. The HSN1 lymphoblasts show 5–10-fold higher levels of DoxSA and ADHD compared to controls. In concordance with this we find, furthermore, highly elevated levels of DoxSA and ADHD in the blood of HSN1 patients.

We therefore conclude that the toxic accumulation of these atypical sphingolipids provides the pathophysiological background for HSN1.

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PO 112

The absence of the peroxiredoxin Pmp20 causes permeabilisation of the peroxisomal membrane and necrotic cell death

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Peroxisomes are important cellular organelles, which contain H_2O_2 producing oxidases together with catalase, which degrades H_2O_2. The presence of catalase in peroxisomes is generally assumed to prevent release of H_2O_2 into the cytoplasm. Peroxisomes, however, also contain other anti-oxidant enzymes, among others peroxiredoxins (Prx's). The physiological function of peroxisomal Prx's is still speculative.

Prx's are involved in the degradation of H_2O_2 and organic hydroperoxides. Prx's have been localized to the cytosol, the endoplasmic reticulum, mitochondria, nuclei and peroxisomes.

The first peroxisomal Prx, Pmp20, was identified in *Candida boidinii* and has glutathione peroxidase activity towards alkyl hydroperoxides and H_2O_2 (Horiguchi et al., 2001).

We identified the Pmp20 homologue of the yeast *Hansenula polymorpha* and analyzed its function *in vivo*. During growth of *H. polymorpha* on methanol massive amounts of H_2O_2 are produced in peroxisomes. We show that cells of a *H. polymorpha PMP20* disruption strain (*pmp20*) have a severe growth defect on methanol, which is paralleled by permeabilisation of the peroxisomal membrane and leakage of peroxisomal matrix proteins into the cytosol.

Methanol-induced *pmp20* cells accumulated enhanced levels of lipid peroxidation products. Moreover, the fatty acid composition of methanol induced *pmp20* cells differed relative to WT controls, suggesting an effect on fatty acid homeostasis. Plating assays and FACS-based analysis of cell death markers revealed that *pmp20* cells show loss of clonogenic efficiency and membrane integrity, when cultured on methanol.

We conclude that the absence of the peroxisomal peroxiredoxin leads to loss of peroxisome membrane integrity and necrotic cell death.

Reference

Horiguchi, et al., 2001. J. Biol. Chem. 276, 14279–14288.

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